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Managing Genetic Resources of Lodgepole Pine in West-Central Alberta
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ABSTRACT

Genetic (isozyme) variation was quantified in 57 stands of lodgepole pine from west-central Alberta. In unmanaged (fire-origin, unthinned) stands there were no differences in genetic diversity related to elevation or stand age. Population differentiation was not related to elevation, geographic location, or management prescription. For harvest-origin stands there were no significant differences in genetic diversity related to regeneration method (natural regeneration vs planting), or pre-commercial thinning. Harvest-origin stands had slightly, but significantly, lower average population genetic diversity than unmanaged stands (2 – 15 %). Fire-origin stands had significantly greater genetic diversity than harvest-origin stands of similar age that were naturally regenerated or planted. Harvest-origin (*vs* unmanaged) stands from the high-elevation b ·ɔding region showed a 21% reduction in allelic richness, owing mainly to missing rare alleles. Compared to individuals from unmanaged stands in the high elevation breeding region, 106 individuals representing selections for the tree improvement program for this region showed lower genetic diversity (~ 15 %) and were missing 47 % of all alleles and 78 % of all rare alleles.

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1. Introduction

In long-lived species, like forest trees, genetic diversity is of particular importance to ensure long-term survival, and stability of forest ecosystems, through future environmental variation (Larsen, 1995; Geburek, 1997). Thus, maintenance of genetic diversity in managed stands of forest trees has become an important focus of sustainable forest management (Libby et al., 1969; Jasso, 1970; Richardson, 1970; Boyle, 1991; Ledig, 1992; Namkoong, 1992; Rogers and Ledig, 1996; Gordon, 1996; Friedman and Foster, 1997). There are a number of ways in which forest management may influence genetic diversity of commercial tree species. Forest fragmentation, which inevitably follows clearcut harvesting, can influence gene flow and patterns of genetic diversity (Saunders et al., 1991; Foré et al., 1992; Ellstrand and Elam 1993; Young et al., 1993). Stand- or landscape-level genetic variation could be altered by planting seedlings, thinning, selective harvesting, or through deployment of material from tree improvement programs (Adams, 1981; Brown and Moran, 1981; Knowles, 1985; Yazdani et al., 1985; Yow et al., 1992; Desponts et al., 1993; El-Kassaby, 1995; Buchert et al., 1997).

Knowledge regarding patterns of genetic variation in natural populations is a pre-requisite to understanding the impact of management practices, and to the development of a program for gene conservation. The uncertain nature of future environmental change, and the difficulty in measuring physiological adaptability, make it difficult to focus conservation programs on preservation of adaptive diversity (Larsen, 1995). Isozymes and DNA markers can play an important role in planning gene conservation programs by allowing for identification of source populations, understanding population size and differentiation across the landscape, and documenting impacts of human intervention on genetic diversity (Geburek, 1997).

Lodgepole pine (*Pinus contorta* Douglas *ex* Loudon var. *latifolia* Engelmann) is a shade-intolerant, early successional conifer and an important timber species in western North America (Ying et al., 1985). Previous genetic studies of lodgepole pine have focused on range-wide variation and population differentiation (Yeh and Layton, 1979; Dancik and Yeh, 1983; Yeh et

al., 1985; Yang et al., 1996). Like many temperate conifers lodgepole pine shows high variation within and little differentiation between populations although there is evidence of some population differentiation related to geographic location, latitude or elevation (Illingworth, 1976; Yeh and Layton, 1979; Yeh et al., 1985; Rehfeldt, 1988).

With the cooperation and support of five major forest companies in Alberta, Canada, we conducted an extensive study of isozyme variation in both wild and managed stands of lodgepole pine across a large area in northwestern Alberta, Canada in order to document patterns of genetic variation in natural populations and to examine the effects of forest management upon these. Specifically, our objectives were to:

1) document baseline levels of genetic variation in unmanaged populations of lodgepole pine as related to: a) elevation; b) geographic location; c) stand age;
2) examine the effect of a) forest management (unmanaged *vs* harvest origin); b) regeneration method after harvesting (natural regeneration, planting); and c) stand tending (pre-commercial thinning) on genetic variation of lodgepole pine;
3) compare genetic variation within a group of genotypes selected for the lodgepole pine high elevation breeding region tree improvement program with that found in unmanaged stands.

2. Materials and Methods

2.1. Stand Sampling

It has been estimated that there is a 95% probability per locus of detecting all alleles with a frequency greater than or equal to 0.05 in a population when 30 diploid individuals per population are sampled (Crawford, 1990); therefore, needle tissue (current year's growth) was collected from 30 trees per stand from 57 lodgepole pine stands over an area of approximately 32,000 km^2 in northwestern Alberta, Canada (Table 1). The stands came from the Forest Management Agreement (FMA) areas of five different forestry companies: Alberta Newsprint Company (Timber), Canadian Forest Products Ltd., Millar Western Forest Products Ltd.,

Weldwood of Canada Ltd. (Hinton Division), and Weyerhaeuser Canada Ltd. (Grande Prairie Operations). For each stand, sampled trees were collected from over a 10 to 20 ha area and were a minimum of 50 m apart. Tissue was packed in ice and immediately shipped to the University of Alberta, where it was transferred to storage at – 70°C. The sampled stands varied in terms of: natural subregion (Alberta Environmental Protection 1994; only identified for stands in the B2 breeding region); breeding region (B1 = low elevation, B2 = high elevation, > 1200 m.a.s.l); stand-origin (fire, harvesting); regeneration method (natural regeneration, planted); and pre-commercial thinning (unthinned, thinned) (Table 1). In addition, we obtained tissue samples from 106 grafted orchard-grown individuals representing the tree improvement selections for the tree improvement program for the B2 (high elevation) breeding region. These individuals represent one tree per stand from each of 106 stands across the FMA areas of Weldwood and Weyerhaeuser. Tree improvement selections were made based on growth rate, growth form, and being free of disease or insect attack.

2.2. Extraction and electrophoresis of enzymes

Standard methods for horizontal starch gel electrophoresis (Soltis et al., 1983) were used to assess genetic variation in the sampled individuals of lodgepole pine. The method for preparing the needle tissue for electrophoresis was modified from Mitton et al., (1979). The centrifugation step was eliminated and, instead, the supernatant was directly absorbed onto filter paper wicks then stored at –70°C. Prior to electrophoresis, wicks were thawed and loaded onto 12% starch gels.

A preliminary study was conducted to determine which buffer systems and enzymatic assays provided optimal activity and resolution. Due to poor resolution, inconsistent banding patterns, and/or uninterpretable banding patterns, menadione reductase (MNR), acid phosphatase (ACP), aldolase (ALDO), and esterase (EST) were not used.

For our study, the resolution of eight enzymes was accomplished using three different gel/electrode buffer systems. The following system was used to resolve malate dehydrogenase

(MDH), malic enzyme (ME), shikimic dehydrogenase (SKD), and 6-phosphogluconate dehydrogenase (6-PGD): electrode buffer of 0.125 M Tris and 1.0 M citric acid, anhydrous (pH 7.0) and a gel buffer of 0.05 M L-histidine-HCl, 1.40 mM EDTA and 1.0 M Tris (pH 7.0) (King and Dancik, 1983). Phosphoglucomutase (PGM), isocitrate dehydrogenase (IDH), and glutamate dehydrogenase (GDH) were resolved with: an electrode buffer of 0.065 M L-histidine (free base) and 0.007 M citric acid monohydrate (pH 6.5) and a gel buffer consisting of a 1:3 ratio of electrode buffer and distilled water (modified from Cardy et al.,1981). Phosphoglucoisomerase (PGI) was resolved with the following system: gel buffer of one part 0.038 M lithium hydroxide monohydrate - 0.188 M boric acid (pH 8.2) and nine parts 0.045 M Tris - 0.007 M citric acid (pH 8.4); the electrode buffer consisted of only the lithium borate component (Soltis et al.,1983).

Electrophoresis for the histidine system was conducted at 75 mA for the first hour and 95 mA for the next four hours. The histidine-citrate system was run continuously at 45 mA for five hours and the lithium-borate system was run at 50 mA for the first hour then 60 mA for the following four hours. All gels were run at 4^{0}C and this temperature was maintained by placing crushed ice on the gels.

Enzymatic assays followed Soltis et al. (1983) with some quantitative modifications. For enzymes encoding more than one isozyme, the most anodally migrating isozyme was designated as 1, the next as 2, and so forth. Similarly, the most anodal allozyme was designated as a, then b, etc. Genetic interpretation of isozymes and allozymes was based on the segregation of bands among individuals in the populations sampled. Comparisons with assays of lodgepole pine megagametophytes, information from previous studies of lodgepole pine (Dancik and Yeh, 1983; Yeh and Layton, 1979), and other plant species (Gottlieb, 1981; Kephart, 1990; King and Dancik, 1983; El-Kassaby, 1981; Sharma and von Wuehlisch, 1998) regarding the subunit structure and the number of isozymes was also used to assist with interpretation.

2.3. Data Analysis

The computer program POPGENE (Yeh et al., 1997) was used to calculate the following diversity statistics for each stand (population) of lodgepole pine: expected heterozygosity [H_{exp}, Nei's unbiased (Nei, 1978)]; observed heterozygosity (H_{obs}); observed average number of alleles per locus (n_a); effective number of alleles per loci (n_e; Hartl and Clark, 1989); and percent polymorphic loci (%Poly, frequency of most common allele $\leq$ 0.99; Gottlieb, 1981). Using the 'multiple populations' option the following parameters were calculated for all individuals in groups of stands and for the B2 tree improvement selections: total expected heterozygosity (H_T); total observed heterozygosity (H_O); total average alleles per locus (A); total percent polymorphic loci (%Poly); fixation index [F_{IS}, estimate of heterozygote deficiency or excess, (Hartl and Clark, 1989)]; and the proportion of variation due to population differentiation (F_{ST}; Hartl and Clark, 1989). We produced an estimated standard error for the B2 selections by bootstrapping (20 groups of 30 randomly selected individuals) in order to facilitate comparison with average population values. Genetic identities [I, Nei's unbiased (Nei, 1978)] were calculated for all pair-wise comparisons of stands and then averaged to show relationships within and among groups of stands based on natural subregion, breeding region, or disturbance type. Geographic distances were calculated by triangulation using latitude and longitude. The relationship between geographic distance and genetic distance was examined using simple correlation analysis.

Analysis of variance (ANOVA) using the SAS software package was used to test for differences in average stand genetic diversity (H_{exp}, H_{obs}, n_a, n_e and %Poly) as follows:

a) as related to breeding region (B1 *vs* B2) or natural subregion (subalpine, upper foothills, B1 breeding region) and age (<10, ~30, >100), using fire origin-unthinned stands;

b) as related to regeneration method (natural regeneration, planting) and age, using harvest origin-unthinned stands;

c) as related to stand origin (fire, harvest) and thinning, using fire- and harvest-origin, natural regeneration, stands of age ~ 30 years;

d) as related to breeding region and management treatment (unmanaged *vs* harvest-origin) using i) all fire-origin unthinned and all harvest-origin stands; and ii) age <10 and ~30 fire-origin unthinned and harvest-origin stands;

e) as related to the combination of stand origin and regeneration method (fire-natural regen *vs* harvest-natural regen *vs* harvest-planted) using fire-origin unthinned and harvest-origin unthinned stands, <10 and ~ 30 year old.

The following is an example of the general linear model used for analysis a) and shows the general form of model used for each analysis:

$$[1] \qquad Y_{ijk} = \mu + R_i + A_j + RA_{ij} + e_{k(ij)}$$

where Y_{ijk} = the observation, μ = the overall mean, R_i = the ith breeding region (B1 or B2) or natural subregion [subalpine, upper foothills or low elevation (B1)], A_j = the jth age (100+, ~30, < 10), RA_{ij} = the interaction of region and age, and $e_{k(ij)}$ = random error associated with the stands within each of the treatment combinations. All main effects were considered to be fixed. Differences were considered significant at $p \leq 0.05$. For analysis e) significant main effects in the ANOVA were followed by Student-Neuman-Keuls multiple range testing.

Allelic richness and the presence of common and rare (frequency < 0.05) alleles were tallied for populations grouped by stand type. Alleles were also categorized as widespread or localized (present in < 25% of all stands; Marshall and Brown, 1975).

Principal Components Analysis (PCA) ordination (using CANOCO; Ter Braak, 1988) was used to examine relationships among populations based on multilocus allele frequencies.

3. Results

The eight enzyme systems assayed were coded by 13 putative loci. Three isozymes, MDH-1, ME-2 and SKD-2, were excluded from our analysis due to poor or inconsistent resolution. Three loci (MDH-2, GDH-1, and PGI-1) were monomorphic in all populations. The remaining 10 loci (MDH-3, MDH-4, ME-1, SKD-1, 6PGD-1, 6PGD-2, PGM-1, PGM-2, IDH-1, and PGI-2) were polymorphic in at least one population (Table 2). A total of 54 allozymes were associated with the 13 isozymes.

For unmanaged (fire-origin unthinned) stands there were no significant differences in mean population genetic diversity (expected heterozygosity, H_{exp}; observed heterozygosity, H_{obs}; observed (n_a) or effective (n_e) number of alleles; or percent polymorphic loci, % poly) between breeding regions, among natural subregions, or among ages (Table 3). There were no significant differences in mean population genetic diversity (any variable) related to age or regeneration method (harvest-origin stands) or stand origin or thinning (fire- or harvest-origin stands) (Table 3).

Unmanaged (fire-origin, unthinned, all ages) stands had significantly greater H_{exp}, H_{obs} and n_e than harvest-origin stands (Table 4, Figure 4). When only fire-origin stands aged <10 and ~30 were included the unmanaged stands still had significantly greater H_{exp} and n_e. There was no significant effect of region or region by management treatment interaction. Fire-origin (natural regeneration) stands had significantly greater H_{exp} and n_e than harvest-origin, planted stands (Table 5, Figure 5).

Harvest-origin stands had lower total expected (H_T, 6 % less), and observed (H_O, 9 % less) heterozygosity, total observed number of alleles per locus (A, 3.83 *vs* 4.00) and % polymorphic loci (%Poly, 67 % *vs* 75 %) than unmanaged (fire-origin, unthinned) stands (Table 6). When subdivided by breeding region this trend of lower genetic diversity in harvest-origin stands held only for the high elevation (B2) breeding regions, from which the majority of stands were sampled.

As a group, the 106 individuals from the B2 region tree improvement program showed lower H_T (15 %), H_O (15 %), A (2.25 *vs* 4.0), and %Poly (42 % *vs* 75 %) than all the unmanaged stands from the B2 region as a group (Table 6). Compared to the average population values for unmanaged stands in the B2 region, the B2 selections showed lower expected (6.4 %) and observed heterozygosity (14.4 %), and the estimated standard errors for the B2 selections would suggest these differences are significant (Tables 4, 6). In contrast, the B2 selections had higher alleles per locus and % polymorphic loci than the average population value for unmanaged stands in the B2 region (Tables 4, 6).

The F_{ST} values showed that only a small proportion (7 to 15 %) of genetic variation was due to population differentiation (Table 6). The results suggest that overall, and for each breeding region, there was slightly greater divergence among harvest-origin stands than among fire-origin stands. The fixation index showed a slight degree of inbreeding (Table 6).

The vast majority of alleles were either, widespread and common (23/51; present in > 25% of all stands, allele frequency > 5%), or localized and rare (22/51) (Table 7). There were eight alleles (seven of them rare) that were not found in any stands from the B1 region. In general, alleles found in the harvest-origin stands were good representations of those found in unmanaged stands from the same breeding region. Harvest-origin stands in the B2 region showed a 21 % reduction in allelic richness compared to unmanaged stands from the same region, owing mainly to a large number of missing rare alleles (Table 7). Unmanaged stands from the B1 region showed lower allelic richness (larger number of missing rare alleles) than harvest-origin stands, but there were far fewer unmanaged stands sampled for the B1 region. There was a large number of missing alleles in the tree improvement selections of the B2 tree improvement material (47 % of all alleles, 78 % of rare alleles) (Table 7).

Genetic identities were high within regions, between subregions within the B2 breeding region, between the two breeding regions, and between harvest-origin and unmanaged stands within each breeding region (Table 8). Genetic identities among harvest-origin stands were

slightly lower than among unmanaged stands within each breeding region, reflecting the trends shown by F_{ST} values (above). There was no relationship between geographic distance and genetic identity among fire-origin stands ($r^2 < 0.0001$).

The ordination results confirmed that there was little population differentiation related to elevation (natural subregion or breeding region) (Figure 1). In general, the B2 tree improvement selections showed a close relationship to unmanaged stands from the B2 region, reflecting similar frequencies of common alleles (Figure 1). In each breeding region, harvest-origin stands (naturally-regenerated and planted) showed close relationships with, and similar spread as, unmanaged stands (Figure 2, 3). Stands clustered to the right on the first PCA axis were characterised by higher than average frequencies of allozymes *Mdh-3*^{a} and *Mdh-4*^{c}; this was the case for both managed and unmanaged stands in each region.

4. Discussion

4.1 Genetic variation in unmanaged populations

We found little relationship between genetic variation and geographic location among stands of lodgepole pine in northwestern Alberta. Other studies of lodgepole pine have shown latitudinal variation among populations over a larger portion of the species' range in western North America (Illingworth, 1976; Yeh and Layton, 1979; Yeh et al., 1985; Rehfeldt, 1988; Yang and Yeh, 1993). Yang and Yeh (1993) found higher heterozygosity in eastern and high altitude populations, but a decline in northern populations. There have been suggestions that genetic variation may be more strongly related to elevational variation at smaller scales (Yeh et al., 1985; Rehfeldt, 1988; Ying and Illingworth, 1986). We found no differences in genetic diversity and no evidence of differentiation related to elevation, as indicated by natural subregion or breeding region, or geographic location and this conforms with studies of morphology and growth for populations in northern Alberta and British Columbia (Ying et al., 1988).

Conifers, in general, show very high levels of genetic diversity (Hamrick et al., 1981). As a result of extensive gene flow and large population size (Epperson and Allard, 1989), many temperate conifers show high levels of within-population genetic variation and little differentiation among populations (Yeh and Layton, 1979; Guries and Ledig, 1981; Yeh, 1981; Hamrick and Godt, 1989). Our values for expected heterozygosity (0.17) are in the range of what has been reported previously for lodgepole pine (0.16; Yeh and Layton, 1979; 0.165; Yeh et al., 1985; 0.194; Yang and Yeh, 1995). We found that 90 to 93 % of variation in unmanaged stands of lodgepole pine was due to diversity within populations; this is slightly lower than what has been found in previous isozyme studies of lodgepole pine in western Canada (96 to 98%; Yeh and Layton 1979; Dancik and Yeh 1983; Yang et al., 1996). The genetic identity values also suggest low levels of population differentiation (I = 0.948 to 0.988) but, surprisingly, values were lower than reported for range-wide studies of lodgepole pine (I = 0.993 to 0.997; Dancik and Yeh, 1983).

These data suggest slight heterozygote deficiencies (F_{IS} = 0.026 to 0.047) and this supports previous isozyme studies on lodgepole pine (F_{IS} = 0.03, 0.06; Dancik and Yeh, 1983; Yeh et al., 1985, respectively). Other wind-pollinated conifers also show heterozygote deficiencies (Guries and Ledig, 1981; Dancik and Yeh, 1983; Knowles, 1991; Wang and Macdonald, 1992). Since lodgepole pine is adapted to regenerate quickly following disturbance, with release of seeds from serotinous cones, stands likely exhibit strong family structure. Apparent inbreeding, then, may arise as a result of outcrossing among related individuals, which established close to one another (Linhart et al., 1981; Sproule and Dancik, 1996).

There is typically little ingress into stands of lodgepole pine following initial establishment, because of its extreme shade intolerance. During the self-thinning phase genetic diversity could decline, due to bottleneck effects, or increase if there was lower mortality of heterozygotes due to greater growth (Mitton et al., 1981). In an old-growth stand of *Pinus sylvestris*, the oldest trees (300 – 400 years) were in Hardy-Weinberg equilibrium while younger trees (100 years)

demonstrated a deficiency of heterozygotes (Tigerstedt et al., 1982). Our results did not show any consistent change in genetic diversity with stand age for lodgepole pine.

4.2 Effects of forest management

Overall our results suggest minor but significant effects of current management practices on genetic diversity of lodgepole pine. Harvest-origin stands showed significantly lower average population expected heterozygosity (2.8 to 15% depending on comparison, Table 4, Figure 4) and observed heterozygosity (4 to 13% depending on comparison, Table 4, Figure 4). These trends were underlain by significantly lower expected heterozygosity in harvested-planted (*vs* fire-naturally regenerated) stands (Figure 5). The observed (non-significant) lower diversity in harvest, natural regeneration (*vs* fire, natural regeneration) stands may be attributed to differences in the release of seeds following the two types of disturbance. Natural regeneration following harvesting depends on release of seeds from serotinous cones on branches spread across the site by drag scarification. Soil surface temperatures may not be sufficient to promote as complete a release of seed as would result from fire.

Our evidence that planted stands have reduced genetic diversity conforms with Gömöry (1992; 13 % reduction for Norway spruce). Other studies (jack pine; Knowles, 1985; Douglas-fir; Adams et al., 1998) and our own previous work on a small number of lodgepole pine stands (Thomas et al., 1999) showed no significant effect of planting on genetic diversity. The bulking of wild seed collections from over a wide geographic area is expected to result in increased genetic diversity (El-Kassaby and Ritland, 1996). However, bottlenecks associated with germination, thinning, and culling, along with sizing of bulked seed-lots have been implicated in loss of genetic diversity during nursery production (Silen and Osterhaus, 1979; El-Kassaby and Thomson, 1996; Stoehr and El-Kassaby, 1997). This inadvertent selection has been implicated as a major cause of reductions in genetic diversity associated with artificial regeneration (El-Kassaby, 1995). Bulking of seed lots may also cause loss of locally-adapted populations (El-

Kassaby and Ritland, 1996; Stoehr and El-Kassaby, 1997). We found no evidence of reduced population differentiation or divergence of allele frequencies in any harvest-origin stands.

Measures of allelic richness may be more useful than heterozygosity for understanding human impacts on genetic diversity because rare alleles contribute little to heterozygosity (Marshall and Brown, 1975; Buchert et al., 1997). Our results show that harvest-origin stands in the B2 region had four times as many missing alleles as unmanaged stands. In the B1 region the reverse was the case but far fewer unmanaged stands were sampled. The tree improvement selections from the B2 tree improvement material showed dramatically lower allelic richness than unmanaged or harvest-origin stands.

The lower levels of heterozygosity in the B2 tree improvement selections were not large (6% *vs* average unmanaged stand, 15 % *vs* total for unmanaged stands), but they, and the dramatic reductions in allelic richness, were unexpected. When seed orchards are comprised of trees selected from over a wide geographic area increased diversity is expected (El-Kassaby and Ritland, 1996). Our results support a few other studies, which have shown that seed orchards and artificial breeding populations have lower genetic diversity (Brown and Moran, 1981; Williams et al., 1995) and allelic richness (Brown and Moran, 1981; Cheliak et al., 1988; Stoehr and El-Kassaby, 1997) in seed orchards and artificial breeding populations. In contrast, there is ample evidence that seed orchards can have greater genetic diversity than natural populations (Bergmann and Ruetz, 1991; Chaisurisri and El-Kasssaby, 1994). Other studies have shown that seed orchards or tree improvement selections have similar genetic diversity to wild populations (Szmidt, 1987; Muona and Harju, 1989; El-Kassaby, 1992; Yow et al., 1992; El-Kassaby and Ritland, 1996; Stoehr and El-Kassaby, 1997).

The effectiveness of *ex situ* collections in capturing *in situ* allelic variation depends upon the distribution of alleles among natural populations (Marshall and Brown, 1975; Yang and Yeh, 1992). Common, widespread alleles are easily captured while common, localized (present in $\leq$ 25 % of populations) alleles require sampling a large number of stands. Capture of rare ($\leq$ 5 %

average frequency per population), widespread alleles is accomplished by more intensive sampling within populations while rare, localized alleles require intensified sampling within and among populations (Yang and Yeh, 1992). Our populations of lodgepole pine showed a preponderance of rare, localized alleles (43 %). This is unusual for wind-pollinated trees (Brown and Moran, 1981) but has been demonstrated previously for lodgepole pine (Yeh and Layton, 1979; Dancik and Yeh, 1983). Almost all alleles missing from the B2 tree improvement selections were in this category (18/23). It will be very difficult to develop a regime for clonal sampling that could capture these alleles and still be consistent with the aims of tree improvement. A reasonable distribution of populations in an *in situ* conservation program, and/or extensive and intensive collection of seed for inclusion in a seedling orchard or gene bank, would be required (Rogers and Ledig, 1996; Ledig et al., 1998).

It has been suggested that very small numbers of clones are appropriate to balance risk and gain in clonal plantations (7 to 25; Libby, 1982; 30 to 40; Roberds and Bishir, 1997) or seed orchards (50 to 60; Kitzmiller, 1990). This may ensure similar heterozygosity to natural stands but allelic richness, perhaps, requires more careful consideration. If we assume rare alleles are deleterious (Adams et al., 1998) their loss is of little concern in a tree improvement program. The genetic variation observed in natural stands of forest trees today is a reflection of the cumulative effect of a long, and on-going, history of selection, mutation, gene flow, postglacial migration, and random drift (Geburek, 1997). A comprehensive program of gene conservation aims to preserve all diversity.

5. References

Adams, W.T. 1981. Population genetics and gene conservation in pacific northwest conifers. *In* Evolution Today, Proceedings of the Second International Congress of Systematic and Evolutionary Biology, July 1980, Vancouver, BC,. *Edited by* G.G.E .Scudder, J.L.Reveal. Hunt Institute for Botanical Documentation, Carnegie-Mellon University, Pittsburgh, Penn. pp. 401-415.

Adams, W.T., Zuo, J., Shimizu, J.Y., and Tappeiner, J.C. 1998. Impact of alternative regeneration methods on genetic diversity in coastal Douglas-fir. For. Sci. **44**: 390-395.

Alberta Environmental Protection. 1994. Natural regions and subregions of Alberta. 1:1,000,000 scale map. Land Information Services, Edmonton, Alta.

Bergmann, F., and Ruetz, W. 1991. Isozyme genetic variation and heterozygosity in random tree samples and selected orchard clones from the same Norway spruce populations. For. Ecol. Manage. **46**: 39-47.

Boyle, T.J.B. 1991. Biodiversity of Canadian forests: current status and future challenges. For. Chron. **68**: 444-453.

Brown, A.H.D., and Moran, G.F. 1981. Isozymes and the genetic resources of forest trees. *In* Proceedings of the Symposium on Isozymes of North American Forest Trees and Forest Insects, July 27, 1979, Berkeley, Calif. *Edited by* M.T. Conkle. United States Department of Agriculture-Forest Service, Berkeley, Calif. General Technical Report PSW-48. pp.1-10.

Buchert, G.P., Rajora, O.P., Hood, J.V., and Dancik, B.P. 1997. Effects of harvesting on genetic diversity in old-growth eastern white pine in Ontario, Canada. Conserv. Biol. **11**: 747-758.

Cardy, B.J., Stuber, C.W., and Goodman, M.M. 1981. Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Institute of Statistics Mimeograph Series No. 1317. North Carolina State University, Raleigh, N. Car.

Chaisurisri, K., and El-Kassaby, Y.A. 1994. Genetic diversity in a seed production population vs. natural populations of sitka spruce. Biodivers. Conserv. **3**: 512-523.

Cheliak, W.M., Murray, G., and Pitel, J.A. 1988. Genetic effects of phenotypic selection of white spruce. For. Ecol. Manage. **24**: 139-149.

Crawford, D.J. 1990. Plant Molecular Systematics: Macromolecular Approaches. John Wiley and Sons, Inc., New York, N.Y.

Dancik, B.P., and Yeh, F.C. 1983. Allozyme variability and evolution of lodgepole pine (*Pinus contorta* var. *latifolia*) and jack pine (*Pinus banksiana*) in Alberta. Can. J. Genet. Cytol. **25**: 57-64.

Desponts, M., Plourde, A., Beaulieu, J., and Daoust, G. 1993. Impact de la sélection sur la variabilité génétique de l'épinette blanche au Québec. Can. J. For. Res. **23**: 1196-1202.

El-Kassaby, Y.A. 1981. Genetic interpretation of malate dehydrogenase isozymes in some conifer species. J. Heredity **72**: 451-452.

El-Kassaby, Y.A. 1992. Domestication and genetic diversity – should we be concerned? For. Chron. **68**: 687-700.

El-Kassaby, Y.A. 1995. Evaluation of the tree-improvement delivery system: factors affecting genetic potential. Tree Phys. **15**: 545-550.

El-Kassaby, Y.A., and Ritland, K. 1996. Impact of selection and breeding on the genetic diversity of Douglas-fir. Biodivers. Conserv. **5**: 795-813.

El-Kassaby, Y.A., and Thomson, A.J. 1996. Parental rank changes associated with seed biology and nursery practices in Douglas-fir. For. Sci. **42**: 228-235.

Ellstrand, N.C., and Elam, D.R. 1993. Population genetic consequences of small population size: implications for plant conservation. Ann. Rev. Ecol. Syst. **24**: 217-242.

Epperson, B.K., and Allard, R.W. 1989. Spatial autocorrelation analysis of the distribution of genotypes within populations of lodgepole pine. Genetics **121**: 369-377.

Foré, S.A., Hickey, R.J. Vankat, J.L., Guttman, S.I., and Schaefer, R.L. 1992. Genetic structure after forest fragmentation: a landscape ecology perspective on *Acer saccharum*. Can. J. Bot. **70**: 1959-1668.

Friedman, S.T., and Foster, G.S. 1997. Forest genetics on federal lands in the United States: public concerns and policy responses. Can. J. For. Res. **27**: 401-408.

Geburek, T. 1997. Isozymes and DNA markers in gene conservation of forest trees. Biodivers. Conserv. **6**: 1639-1654.

Gömöry, D. 1992. Effects of stand origin on the genetic diversity of Norway spruce (*Picea abies* Karst.) populations. For. Ecol. Manage. **54**: 215-223.

Gordon, A.G. 1996. The sweep of the boreal in time and space, from forest formations to genes, and implications for management. For. Chron. **72**: 19-30.

Gottlieb, L.D. 1981. Electrophoretic evidence and plant populations. Prog. Phytochem. **7**: 1-46.

Guries, R.P., and Ledig, F.T. 1981. Genetic structure of populations and differentiation in forest trees. *In* Proceedings of the Symposium on Isozymes of North American Forest Trees and Forest Insects, July 27, 1979, Berkeley, Calif. *Edited by* M.T. Conkle. United States Department of Agriculture-Forest Service, Berkeley, Calif. General Technical Report PSW-48. pp. 42-47.

Hamrick, J.L., and Godt, M.J.W. 1989. Allozyme diversity in plant species. *In* Plant Population Genetics, Breeding and Genetic Resources. *Edited by* A.H.D. Brown, M.T. Clegg, A.L.Kahler, and B.S. Weir. Sinauer Associates, Sunderland, Mass. pp. 43-63.

Hamrick, J.L., Mitton, J.B., and Linhart, Y.B. 1981. Levels of genetic variation in trees: influence of life history characteristics. *In* Proceedings of the Symposium on Isozymes of North American Forest Trees and Forest Insects, July 27, 1979, Berkeley, Calif. *Edited by* M.T. Conkle. United States Department of Agriculture-Forest Service, Berkeley, Calif. General Technical Report PSW-48. pp.35-41.

Hartl, D.L., and Clark, A.G. 1989. Principles of Population Genetics, 2nd ed. Sinauer Associates, Sunderland, Mass.

Illingworth, K. 1976. Inter- and intra-provenance variation in heights of three year old *Pinus contorta* Dougl. *In Pinus contorta* Provenance Studies. *Edited by* R. Line. For. Comm. Res. Dev. Pap. No. 114. pp. 98-106.

Jasso, J.M. 1970. Impact of silviculture on forest gene resources. Unasylva **24**: 70-75.

Kephart, S. 1990. Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. Amer. J. Bot. **77**: 693-712.

King, J.N., and Dancik, B.P. 1983. Inheritance and linkage of isozymes in white spruce (*Picea glauca*). Can. J. Genet. Cytol. **25**: 430-436.

Kitzmiller, J.H. 1990. Managing genetic diversity in a tree improvement program. For. Ecol. Manage. **35**: 131-149.

Knowles, P. 1985. Comparison of isozyme variation among natural stands and plantations: jack pine and black spruce. Can. J. For. Res. **15**: 902-908.

Knowles, P. 1991. Spatial genetic structure within two natural stands of black spruce (*Picea mariana* (Mill.) B.S.P.). Silvae Genet. **40**: 13-19.

Larsen, J.B. 1995. Ecological stability of forests and sustainable silviculture. For. Ecol. Manage. **73**: 85-96.

Ledig, F.T. 1992. Human impacts on genetic diversity in forest ecosystems. Oikos **63**: 87-108.

Ledig, F.T., Vargas-Hernández, J.J., and Johnsen, K.H. 1998. The conservation of forest genetic resources: case histories from Canada, Mexico, and the United States. J. For. **96**: 32-41.

Libby, W.J. 1982. What is a safe number of clones per plantation? *In* Resistance to Diseases and Pests in Forest Trees: Proceedings of the Third International Workshop on the Genetics of Host-Parasite Interactions in Forestry, Sept. 14-21, 1980, Wageningen, The Netherlands. *Edited by* B.R.Heybroeck, B.R. Stephen, and K. von Weissenberg. Centre

for Agricultural Publishing and Documentation, Wageningen, The Netherlands. pp. 342-360.

Libby, W.J., Stettler, R.F., and Seitz, F.W.. 1969. Forest genetics and forest-tree breeding. Ann. Rev. Genet. **3**: 469-494.

Linhart, Y.B., Mitton, J.B., Sturgeon, K.B., and Davis, M.L. 1981. Genetic variation in space and time in a population of ponderosa pine. Heredity **46**: 407-426.

Marshall, D.R., and Brown, A.H.D. 1975. Optimum sampling strategies in genetic conservation. *In* Crop Genetic Resources for Today and Tomorrow. *Edited by* O.H. Frankel and J.G. Hawkes. Cambridge University Press, Cambridge, UK. pp. 53-80.

Mitton, J.B., Knowles, P., Sturgeon, K.B., Linhart, Y.B., and Davis, M. 1981. Associations between heterozygosity and growth rate variables in three western forest trees. *In* Proceedings of the Symposium on Isozymes of North American Forest Trees and Forest Insects, July 27, 1979, Berkeley, Calif. *Edited by* M.T. Conkle. United States Department of Agriculture-Forest Service, Berkeley, Calif. General Technical Report PSW-48. pp. 27-34.

Mitton, J.B., Linhart, Y.B., Sturgeon, K.B., Hamrick, J.L. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. J. Heredity **70**: 86-89.

Muona, O., and Harju, O. 1989. Effective population size, genetic variability, and mating system in natural stands and seed orchards of *Pinus sylvestris*. Silvae Genet. **38**: 221-228.

Namkoong, G. 1992. Biodiversity – issues in genetics, forestry and ethics. For. Chron. **68**: 438-443.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**: 583-590.

Rehfeldt, G.E. 1988. Ecological genetics of *Pinus contorta* from the Rocky Mountains (USA): a synthesis. Silvae Genet. **37**: 131-135.

Richardson, S.D., 1970. Gene pools in forestry. *In* Genetic Resources in Plants – their Exploration and Conservation. *Edited by* O.H. Frankel and O.H. Bennet. Blackwell Scientific Publishers, Oxford, UK. pp. 353-365.

Roberds, J.H.,and Bishir, J.W. 1997. Risk analyses in clonal forestry. Can. J. For. Res. **27**: 425-432.

Rogers, D.L., and Ledig, F.T. 1996. The status of temperate North American forest genetic resources. Report No. 16. Genetic Resources Conservation Program, University of California, Davis, Calif.

Saunders, D.A., Hobbs, R.J., and Margules, C.R. 1991. Biological consequences of ecosystem fragmentation: a review. Conserv. Biol. **5**: 18-32.

Silen, R., and Osterhaus, C., 1979. Reduction of genetic base by sizing of bulked Douglas-fir seed lots. Tree Plant. Notes **30**: 24-30.

Sharma, K., and von Wuehlisch, G. 1998. Genetic interpretation of malate dehydrogenase (MDH) isozyme gene loci using a new staining approach and the genetic control of ten other isozymes in *Pinus roxburghii* Sarg. Silvae. Genet. **47**: 321-332.

Soltis, D.E., Haufler, C.H., Darrow, D.C., and Gastony, G.H. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am. Fern. J. **73**: 9-27.

Sproule, A.T., and Dancik, B.P. 1996. The mating system of black spruce in north-central Alberta, Canada. Silvae Genet. **45**: 159-164.

Stoehr, M.U., and El-Kassaby, Y.A. 1997. Levels of genetic diversity at different stages of the domestication cycle of interior spruce in British Columbia. Theor. Appl. Genet. **94**: 83-90.

Szmidt, A. 1987. Genetic composition of seed orchard crops. For. Ecol. Manage. **19**: 227-232.

Ter Braak, C.J.F. 1988. CANOCO – a FORTRAN program for canonical community ordination by partial detrended canonical correspondence analysis, principal components analysis

and redundancy analysis (ver 2.1). Groep Landbouwwiskunde Technical Report: LWA-88-02, Wageningen, The Netherlands.

Thomas, B.R., Macdonald, S.E., Hicks, M., Adams, D.L., and Hodgetts, R.B. 1999. Effects of reforestation methods on genetic diversity of lodgepole pine: an assessment using microsatellite and randomly amplified polymorphic DNA markers. Theor. Appl. Genet. **98**: 793-801.

Tigerstedt, P.M.A., Rudin, D., Niemelä,T. and Tammisola, J. 1982. Composition and neighbouring effect in a naturally regenerating population of Scots pine. Silva Fenn. **16**: 122-129.

Wang, Z.M., and Macdonald S.E. 1992. Peatland and upland black spruce populations in Alberta, Canada: isozyme variation and seed germination ecology. Silvae Genet. **41**: 117-122.

Williams, C.G., Hamrick, J.L., and Lewis, P.O. 1995. Multiple-population versus hierarchical conifer breeding programs: a comparison of genetic diversity levels. Theor. Appl. Genet. **90**: 584-594.

Yang, R.C., Yeh, F.C. 1992. Genetic consequences of *in situ* and *ex situ* conservation of forest trees. For. Chron. **68**: 720-729.

Yang, R.C., and Yeh, F.C. 1993. Multilocus structure in *Pinus contorta* Dougl. Theor. Appl. Genet. **87**: 568-576.

Yang, R.C., and Yeh, F.C. 1995. Patterns of gene flow and geographic structure in *Pinus contorta* Dougl. For. Genet. **2**: 65-75.

Yang, R.C., Yeh, F.C., and Yanchuk, A.D. 1996. A comparison of isozyme and quantitative genetic variation in *Pinus contorta* ssp. *latifolia* by F_{ST}. Genetics **142**: 1045-1052.

Yazdani, R., Muona, O., Rudin, D., and Szmidt, A.E. 1985. Genetic structure of a *Pinus sylvestris* L. seed-tree stand and naturally regenerated understorey. For. Sci. **31**: 430-436.

Yeh, F.C. 1981. Analyses of gene diversity in some species of conifers. *In* Proceedings of the Symposium on Isozymes of North American Forest Trees and Forest Insects, July 27, 1979, Berkeley, Calif. *Edited by* M.T. Conkle. United States Department of Agriculture-Forest Service, Berkeley, Calif. General Technical Report PSW-48. pp. 48-52.

Yeh, F.C., and Layton, C. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* ssp. *latifolia*. Can. J. Genet. Cytol. **21**: 487-503.

Yeh, F.C., Cheliak, W.M., Dancik, B.P., Illingworth, K., Trust, D.C., and Pryhitka, B.A. 1985. Population differentiation in lodgepole pine, *Pinus contorta* ssp. *latifolia*: a discriminant analysis of allozyme variation. Can. J. Genet. Cytol. **27**: 210-218.

Yeh, F.C., Yang, R.C., Boyle, T., Ye, B.J., and Mao, J. 1997. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alta.

Ying, C.C., and Illingworth, K. 1986. Lodgepole pine provenance research in northwestern Canada with particular reference to the Yukon Territory. *In* Provenances and Forest Tree Breeding for High Latitude. *Edited by* Lindgren. Rep. No. 6. Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umea, Sweden. pp. 189-218.

Ying, C.C., Illingworth, K., and Carlson, M. 1985. Geographic variation in lodgepole pine and its implications for tree improvement in British Columbia. *In* Proceedings of the Symposium on Lodgepole Pine: the Species and its Management, Spokane, Wash. and Vancouver, B.C., 1984. Edited by D.M. Baumgartner, R.G. Krebill, J.T. Arnott, and G.F. Weetman. Washington State University Cooperative Extension Service, Pullman, Wash. pp. 45-53.

Ying, C.C., Thompson, C., and Herring, L. 1988. Geographic variation, nursery effect and early selection in lodgepole pine. Can. J. For. Res. **19**: 832-841.

Young, A.G., Merriam, H.G., and Warwick, S.I. 1993. The effects of forest fragmentation on genetic variation in Acer saccharum Marsh. (sugar maple) populations. Heredity **71**: 277-289.

Yow, T.H., Wagner, M.R., Wommack, D.E., and Tuskan, G.A. 1992. Influence of selection for volume growth on the genetic variability of southwestern Pondersosa Pine. Silvae Genet. **41**: 326-333.

Table 1

Summary of information for the sampled lodgepole pine stands: Stand type, breeding region, natural subregion (where identified), disturbance type, company, geographic location, regeneration method, thinning (year if yes), and stand age (years). 30 individuals were collected per stand

F-NR-UT	B2	UF	Fire	WD	53.35.43	117.37.04	Natural	No	> 100
100+	B2	UF	Fire	WD	53.04.06	117.02.05	Natural	No	102
	B2	UF	Fire	WH	54.28.06	119.32.33	Natural	No	140
	B2	UF	Fire	WH	54.00.38	118.37.31	Natural	No	110
	B2	UF	Fire	ANC	54.06.05	117.41.16	Natural	No	100
F-NR-UT	B2	SA	Fire	WD	52.50.49	116.50.48	Natural	No	~157
100+	B2	SA	Fire	WD	53.39.39	118.19.86	Natural	No	~186
	B2	SA	Fire	WH	54.22.26	119.23.35	Natural	No	120
	B2	SA	Fire	WH	54.08.52	119.25.47	Natural	No	130
	B2	SA	Fire	ANC	53.47.42	118.27.27	Natural	No	139
F-NR- UT	B2	UF	Fire	WD	53.05.03	117.23.23	Natural	No	5-7
<10	B2	UF	Fire	WD	53.06.32	117.23.23	Natural	No	7-8
	B2	UF	Fire	WD	53.07.26	117.21.28	Natural	No	6-7
	B2	UF	Fire	WD	53.07.26	117.21.28	Natural	No	6-8
	B2	UF	Fire	WH	54.14.06	119.43.04	Natural	No	16
F-NR- UT	B2	UF	Fire	WD	53.14.38	117.39.49	Natural	No	34-37
~30	B2	UF	Fire	WD	53.48.25	118.06.13	Natural	No	38-41
	B2	UF	Fire	WD	53.56.15	116.29.24	Natural	No	33-37
	B2	UF	Fire	WD	53.54.59	117.44.07	Natural	No	32
	B2	UF	Fire	WH	54.30.32	119.04.33	Natural	No	35
F-NR- TH	B2	UF	Fire	WD	53.14.23	117.22.18	Natural	1975	34-39
~30	B2	UF	Fire	WD	53.14.23	117.22.18	Natural	1962	36-40
	B2	UF	Fire	WD	53.13.58	117.21.27	Natural	1973	39
H-PL- UT	B2	UF	Harvest	WD	53.41.25	117.41.03	Planted	No	8
<10	B2	UF	Harvest	WH	54.22.27	119.07.49	Planted	No	10
	B2	UF	Harvest	WH	54.23.18	119.16.45	Planted	No	8
H-PL- UT	B2	UF	Harvest	WD	53.35.17	117.31.05	Planted	No	~30
~30	B2	UF	Harvest	WD	53.12.14	117.30.12	Planted	No	25-39
	B2	UF	Harvest	WH	54.25.07	119.09.03	Planted	No	23

H-NR- UT	B2	UF	Harvest	WD	53.40.47	117.42.14	Natural	No	5-6
<10	B2	UF	Harvest	WH	54.25.31	118.57.04	Natural	No	8
	B2	UF	Harvest	WH	54.21.18	118.57.23	Natural	No	12
H-NR- UT	B2	UF	Harvest	WD	53.35.44	117.29.37	Natural	No	~30
~30	B2	UF	Harvest	WD	53.13.39	117.32.05	Natural	No	34
	B2	UF	Harvest	WH	54.28.32	118.57.03	Natural	No	23
	B2	UF	Harvest	WH	54.27.27	118.58.53	Natural	No	24
H-NR- TH	B2	UF	Harvest	WD	53.42.41	117.26.44	Natural	1981	33
~30	B2	UF	Harvest	WD	53.43.38	118.11.03	Natural	1980	36
	B2	UF	Harvest	WD	53.13.59	117.31.04	Natural	1977	33
H-NR- TH	B1		Harvest	WD	53.14.37	116.36.06	Natural	No	36
~30	B1		Harvest	CF	54.33.02	117.51.44	Natural	No	21
H-NR- UT	B1		Harvest	ANC	54.04.20	116.39.40	Natural	No	8
<10	B1		Harvest	ANC	54.04.20	116.39.40	Natural	No	8
H-NR- UT	B1		Harvest	WH	54.37.56	118.49.47	Natural	No	~30
~30	B1		Harvest	CF	54.38.37	117.56.27	Natural	No	~27
H-PL- UT	B1		Harvest	MW	54.08.55	115.57.46	Planted	No	3
<10	B1		Harvest	ANC	54.03.24	116.38.16	Planted	No	8
H-PL- UT	B1		Harvest	WH	54.37.56	118.49.47	Planted	No	~30
~30	B1		Harvest	CF	54.36.02	117.54.00	Planted	No	29
F-NR- UT	B1		Fire	CF	54.33.47	118.08.35	Natural	No	2-4
<10	B1		Fire	ANC	54.04.19	117.36.46	Natural	No	4
F-NR- UT	B1		Fire	WH	54.29.67	118.58.28	Natural	No	24
~30	B1		Fire	MW	54.01.16	116.27.07	Natural	No	~40
F-NR- TH	B1		Fire	WH	54.30.44	118.58.03	Natural	1982	27
~30	B1		Fire	MW	54.01.14	116.18.11	Natural	1982	~40
F-NR- UT	B1		Fire	WD	53.18.45	116.40.56	Natural	No	106
100	B1		Fire	WH	54.37.56	118.49.47	Natural	No	> 100

[a] disturbance type (F = fire, H = harvesting) – Regeneration method (NR = natural regeneration, P = planting) – thinning (UT = unthinned, TH = thinned) – approximate age

[b] B2 = high elevation pine breeding region (>1200 m.a.s.l.), B1 = low elevation pine breeding region (<1200 m.a.s.l.)

[c] natural subregion (Alberta Environmental Protection 1994) when identified: UF = Upper Foothills, SA = Subalpine

[d] company (ANC = Alberta Newsprint, CF = Canadian Forest Products, MW = Millar Western, WD = Weldwood, WH = Weyerhaeuser)

[e] degrees.minutes.seconds

Table 2

Isozymes assayed in lodgepole pine, including the number of allozymes and subunit composition

Isozyme	Number of Allozymes	Subunit Composition[a]
MDH-2	1	
MDH-3	7	D
MDH-4	7	
ME-1	3	T
SKD-1	5	M
6PGD-1	9	D
6PGD-2		
PGM-1	5	M
PGM-2		
IDH-1	5	D
GDH-1	1	H
PGI-1	1	D
PGI-2	10	

[a] M, monomer; D, dimer; T, tetramer; H, hexamer

Table 3

Means (average stand value and standard error (s.e.)) of various genetic diversity parameters for stands grouped by: natural subregion and breeding region, age, regeneration method, thinning. There were no significant main effects or interactions in the analyses of variance

Breeding Region and Natural Subregion							
(fire-unthinned, all ages)[f]							
Upper Foothills	15	mean	0.173	0.158	1.961	1.436	38.9
(B2 breeding region)		s.e.	0.007	0.009	0.047	0.017	1.94
Sub-Alpine	5	mean	0.176	0.180	1.933	1.415	43.3
(B2 breeding region)		s.e.	0.017	0.025	0.143	0.033	6.68
B1 breeding region	6	mean	0.174	0.166	1.842	1.487	33.3
		s.e.	0.007	0.010	0.034	0.025	2.15
Age							
(fire-unthinned, all regions)[f]							
100+ yrs	12	mean	0.176	0.175	1.972	1.424	39.6
		s.e.	0.010	0.013	0.075	0.020	3.27
~ 30 yrs	7	mean	0.169	0.153	1.905	1.450	40.5
		s.e.	0.007	0.010	0.042	0.030	2.17
< 10 yrs	7	mean	0.175	0.157	1.876	1.471	34.5
		s.e.	0.008	0.010	0.052	0.022	2.84
(harvest-unthinned, natural regen, all regions)[f]							
< 10 yrs	5	mean	0.161	0.141	1.856	1.415	31.7
		s.e.	0.010	0.013	0.105	0.030	4.86
~ 30 yrs	6	mean	0.160	0.133	1.828	1.450	35.0
		s.e.	0.009	0.030	0.068	0.046	4.08
(harvest-unthinned, planted, all regions)[f]							
< 10 yrs	5	mean	0.153	0.135	1.883	1.389	33.3
		s.e.	0.006	0.005	0.082	0.025	0.00
~ 30 yrs	5	mean	0.146	0.142	1.783	1.366	36.7
		s.e.	0.009	0.008	0.073	0.021	4.25

Regeneration Method							
(harvest-unthinned, 10, 30 yr, all regions)[f]							
Natural regeneration	11	mean	0.160	0.137	1.834	1.424	32.6
		s.e.	0.006	0.014	0.054	0.025	2.86
Planted	10	mean	0.150	0.139	1.833	1.378	35.0
		s.e.	0.005	0.005	0.054	0.016	2.08
Thinning							
(harvest, natural regen, ~ 30 yrs, all regions)[f]							
Unthinned	6	mean	0.160	0.133	1.828	1.450	35.0
		s.e.	0.009	0.030	0.068	0.046	4.08
Thinned	5	mean	0.168	0.168	2.000	1.445	36.7
		s.e.	0.016	0.023	0.151	0.066	2.04
(fire, natural regen, ~ 30 yrs, all regions)[f]							
Unthinned	7	mean	0.169	0.153	1.905	1.450	40.5
		s.e.	0.007	0.010	0.042	0.030	2.17
Thinned	5	mean	0.166	0.142	1.883	1.443	36.7
		s.e.	0.006	0.010	0.086	0.030	2.04

[a] average population expected heterozygosity (Nei 1978)
[b] average population observed heterozygosity
[c] average population observed # of alleles
[d] average population effective number of alleles (Hartl and Clark 1989)
[e] average population percent polymorphic loci
[f] in brackets: stand types included in the averages given

Table 4

Means (average stand value and s.e.) of various genetic diversity parameters for unmanaged (fire origin-unthinned) *vs* harvest-origin, stands, overall and divided by breeding region

Unmanaged (all ages)	26	mean	0.174	0.164	1.928	1.444	38.5
		s.e.	0.005	0.007	0.039	0.014	1.80
			0.027[f]	***0.020***		***0.021***	
Unmanaged (10, 30 yrs)	14	mean	0.172	0.155	1.891	1.461	37.5
		s.e.	0.005	0.007	0.032	0.018	1.90
			0.018[g]			***0.022***	
Harvest-origin (10, 30 yrs)	26	mean	0.158	0.144	1.866	1.410	34.3
		s.e.	0.005	0.008	0.042	0.018	1.49
B1 unmanaged (all ages)	6	mean	0.174	0.166	1.842	1.487	33.3
		s.e.	0.007	0.010	0.034	0.025	2.15
B1 unmanaged, (10, 30 yrs)	4	mean	0.181	0.171	1.846	1.513	33.3
		s.e.	0.008	0.014	0.054	0.020	3.40
B1 harvest-origin (10, 30 yrs)	10	mean	0.169	0.146	1.953	1.443	35.8
		s.e.	0.009	0.019	0.088	0.033	2.79
B2 unmanaged (all ages)	20	mean	0.173	0.160	1.939	1.433	39.1
		s.e.	0.006	0.008	0.045	0.013	1.92
B2 unmanaged (10, 30 yrs)	10	mean	0.168	0.148	1.908	1.440	39.2
		s.e.	0.006	0.008	0.040	0.021	2.17
B2 harvest-origin (10, 30 yrs)	16	mean	0.150	0.142	1.811	1.390	33.3
		s.e.	0.004	0.005	0.037	0.019	1.70

[a, b, c, d, e] as in Table 3

[f] significant difference (p): unmanaged *vs* harvest-origin in analysis of variance including all ages of unmanaged stands. There were no significant Origin by Region interactions.

[g] significant difference (p): unmanaged vs harvest-origin in analysis of variance including only stands aged <10 and ~30 yrs.

See also Figure 4.

Table 5

Means (average stand value and s.e.) of various genetic diversity parameters for three stand types [fire-natural regeneration, harvested-natural regeneration, harvested-planted (ages <10 and ~30 yrs for each)]

F-NR	14	mean	0.155	1.89	37.5
		s.e.	0.007	0.032	1.91
H-NR	11	mean	0.137	1.833	32.6
		s.e.	0.014	0.054	2.85
H-PL	10	mean	0.138	1.833	35.0
		s.e.	0.005	0.054	2.08

[a] Fire-natural regeneration (F-NR), Harvest-natural regeneration (H-NR), and Harvest-planted (H-PL) (ages <10 and ~ 30 yrs for all)

[b, c, d] as in Table 3

See also Figure 5.

Table 6

Total genetic variation within groupings of stand types (total population estimates) for unmanaged (fire origin-unthinned) *vs* harvest origin stands (overall and divided by breeding region).

	[illegible]						
All unmanaged	26 (780)	0.189	0.162	4.00	75.0	0.110	0.047
All harvest-origin	26 (780)	0.178	0.148	3.83	67.0	0.145	0.037
B1 region unmanaged	6 (180)	0.182	0.163	2.50	50.0	0.071	0.026
B1 harvest-origin	10 (300)	0.189	0.162	3.25	58.0	0.131	0.025
B2 region unmanaged	20 (600)	0.190	0.162	4.00	75.0	0.105	0.045
B2 harvest-origin	16 (480)	0.172	0.142	3.17	67.0	0.145	0.040
B2 tree improvement selections	1 (106)	0.162 (0.002)[g]	0.137 (0.002)	2.25 (0.023)	41.7 (0.683)		

[a] total expected heterozygosity based on all individuals in these populations
[b] total observed heterozygosity based on all individuals in these populations
[c] total average alleles per locus based on all individuals in these populations
[d] total percent polymorphic loci based on all individuals in these populations
[e] proportion of genetic variability that is due to population differentiation
[f] deficiencies of heterozygotes relative to Hardy-Weinberg expectations
[g] estimated standard error (based on 20 randomly selected groups of 30 individuals)

Table 7

a) Summary of alleles found at polymorphic loci in different groupings of stand types: unmanaged (fire-unthinned) *vs* harvest-origin [subdivided by breeding region (B1 low elevation, B2 high elevation)], B2 tree improvement selections, and thinned *vs* unthinned (fire or harvest origin, natural regeneration, age ~30 yrs). b) breakdown of number of alleles by occurrence in populations (localized: present in < 25 % of populations) and frequency within populations (rare = frequency < 0.05).

a)

Stand type	Total # of alleles	# Popns (Indiv/popn)	Common alleles missing	Rare alleles missing
B2 Unmanaged	48	20 (30)	0	3
B2 Harvest	38	16 (30)	1	12
B2 Tree Improvement	27	1 (106)	6	18
B1 Unmanaged	30	6 (30)	4	17
B1 Harvest	39	10 (30)	2	10
Thinned	40	10 (30)	3	8
Unthinned	41	13 (30)	1	9

Total possible (all stands)	51
Total rare (all stands)	23

b)

Total # alleles	Widespread, common	Localized, common	Widespread, rare	Localized, rare
51	23	5	1	22

Table 8

Average genetic identity (Nei's unbiased I, Nei 1978) within and between groups of stand types: breeding region (B1 = low elevation, B2 = high elevation), natural subregion (UF = upper foothills, SA = subalpine, Alberta Environmental Protection 1994), disturbance type (fire, harvest).

Grouping	Average I
Within B1 (fire)	0.9484
Within B1 (harvest)	0.9291
B1 fire *vs* harvest	0.9449
Within UF (fire)	0.9792
Within SA (fire)	0.9642
UF *vs* SA (fire)	0.9729
Within B2 (fire)	0.9759
Within B2 (harvest)	0.9633
B2 fire *vs* harvest	0.9684
B1 *vs* B2 (fire)	0.9633
B1 *vs* B2 (harvest)	0.9449

Figure 1. Results of ordination (principle component analysis) of allele frequencies of fire-origin lodgepole pine stands for the B1 (low elevation) and B2 (high elevation) breeding regions including thinned and unthinned stands, as well as the B2 tree improvement selections. Separation of stands on the first and second principle component axes.

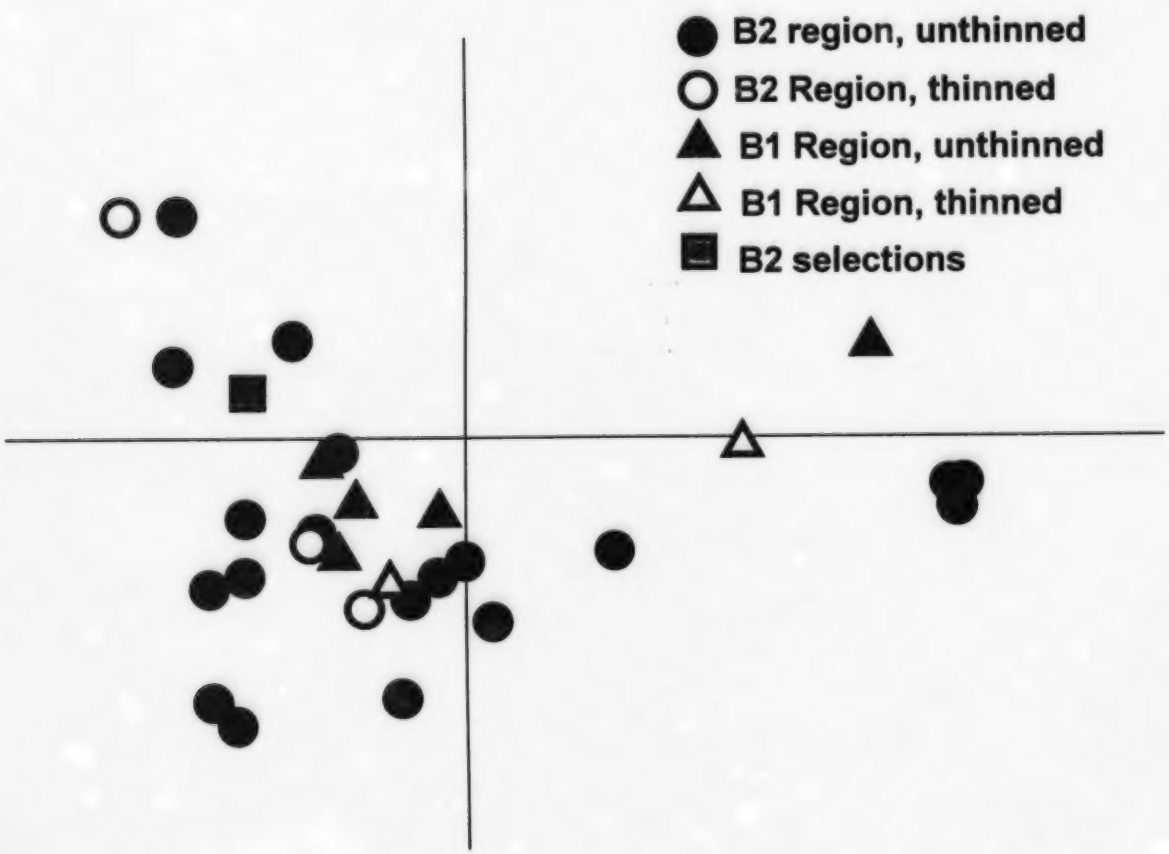

Figure 2. Results of ordination (principle component analysis) of allele frequencies of all sampled lodgepole pine stands from the B2 (high elevation) breeding region including fire- and harvest- origin stands, naturally regenerated and planted stands, and thinned and unthinned stands. Separation of stands on the first and second principle component axes.

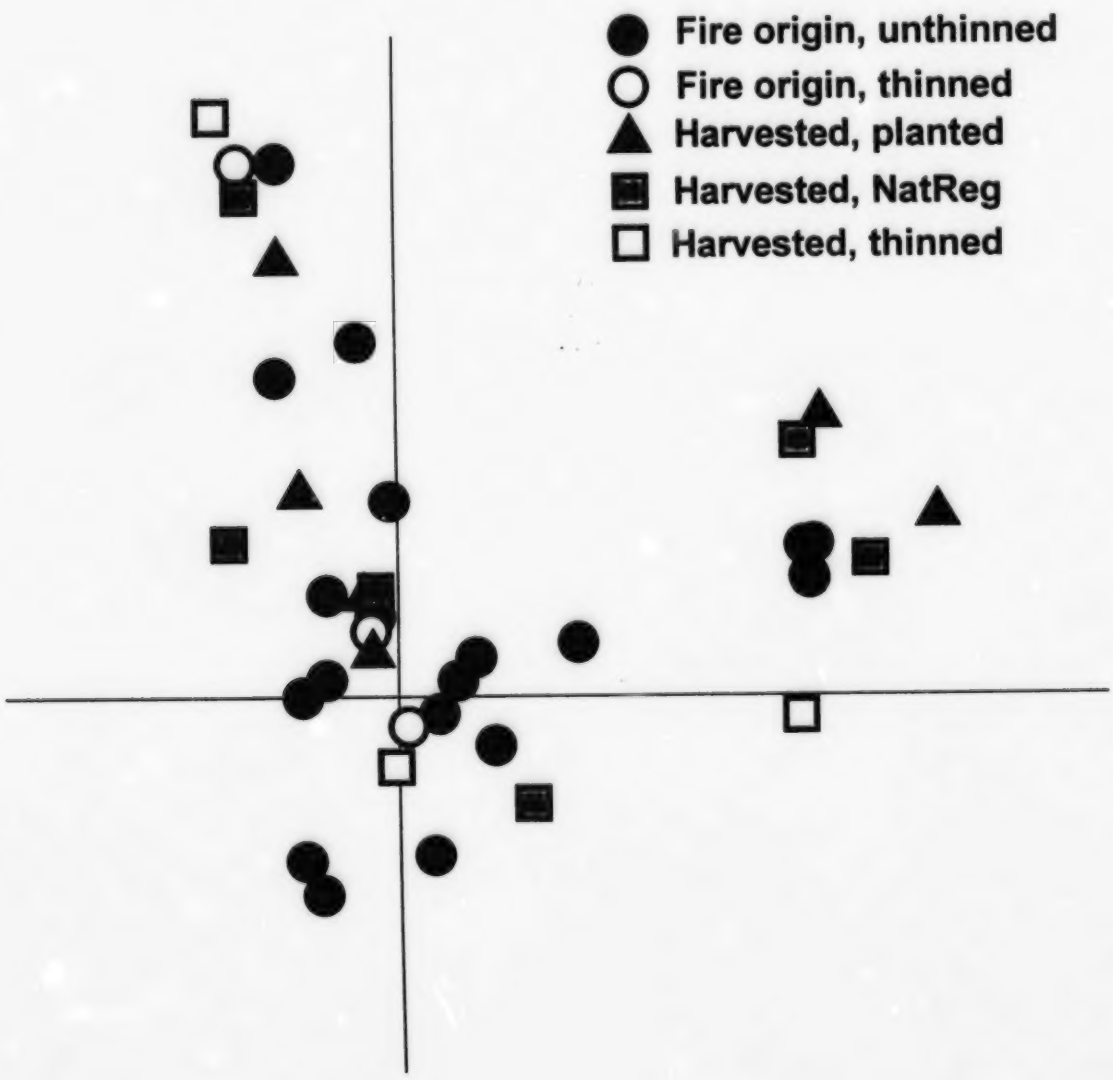

Figure 3. Results of ordination (principle component analysis) of allele frequencies of all sampled lodgepole pine stands from the B1 (low elevation) breeding region including fire- and harvest- origin stands, naturally regenerated and planted stands, and thinned and unthinned stands. Separation of stands on the first and second principle component axes.

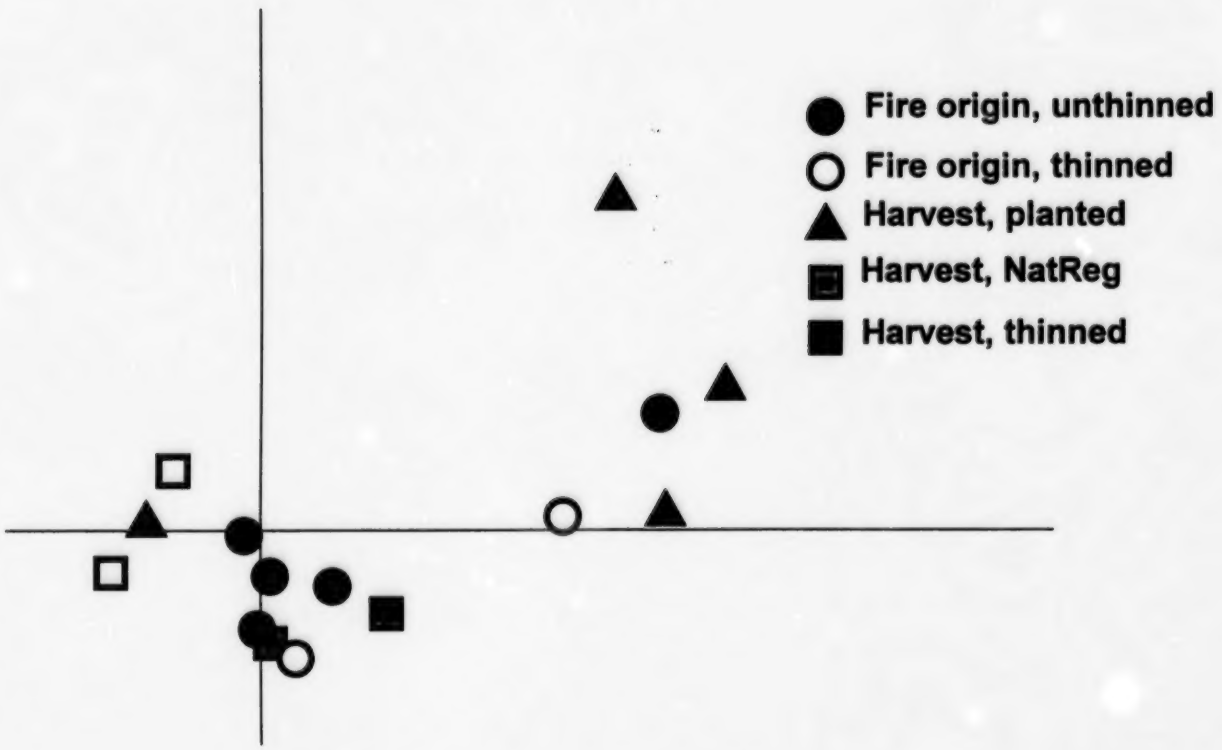

Figure 4. Average population heterozygosity (expected and observed) and effective number of alleles for managed *vs* unmanaged populations of lodgepole pine (average across regions). p values indicate significant differences from managed populations.

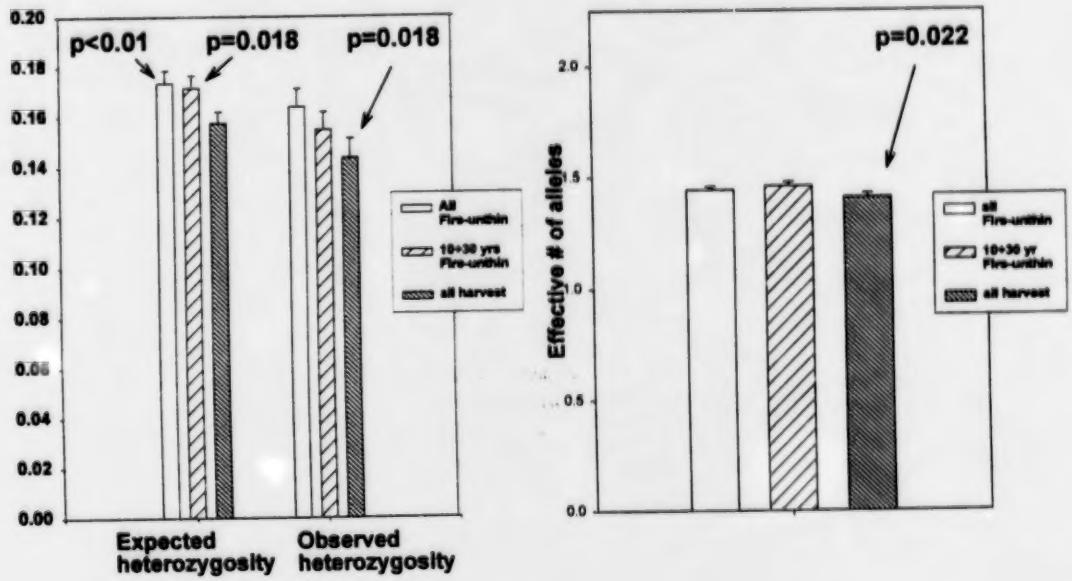

Figure 5. Genetic diversity as related to stand origin and regeneration method for unthinned stands aged ~ 10 + ~ 30 years. p values indicate significant difference among the three categories (by ANOVA). Bars with different letters are significantly different (by SNK test).